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Using GIS to Predict Cetacean Strandings Related to Harmful Algal Blooms

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HALMOS COLLEGE OF NATURAL SCIENCES AND OCEANOGRAPHY

USING GIS TO PREDICT CETACEAN STRANDINGS RELATED TO HARMFUL ALGAL BLOOMS

By

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Abstract

Exposure to harmful algal bloom (HAB) toxins, such as *Karenia brevis*, has been linked to cetacean strandings and mortalities. Biological and environmental data from a bottlenose dolphin (*Tursiops truncatus)* Unusual Mortality Event (UME) in 2004 are compared to those from pilot whale *(Globicephala macrorhynchus)* mass stranding events in 2013-2014 in western Florida. Geographical Information Systems (GIS) maps have been created by overlaying stranding locations and select *K. brevis* presence data in order to identify common spatial and temporal trends. Preliminary results indicate that elevated *K. brevis* levels (>10,000 cells/L) in Sarasota Bay during January-February and November may be predictive of stranding events in the following 2-9 weeks for bottlenose dolphins and pilot whales, respectively. Further development and refinement of this model to project the timing and location of potential stranding events may allow conservation managers and first responders to proactively stage equipment, accelerate response times, and increase live mammal rescues.

Key Words

Karenia brevis, Geographical Information Systems, pilot whales, *Globicephala macrorhynchus*, bottlenose dolphins, *Tursiops truncatus*, marine mammal, mass stranding, UME, Unusual Mortality Event, species conservation, rescue response

Introduction

Harmful Algal Blooms

Harmful Algal Blooms (HABs) are created by aquatic species that have the ability to cause harm to either aquatic or terrestrial organisms through the production of various toxins (Landsberg 2002). The toxicity, demographic location, and manner of interspecies interactions can vary by toxin type. These blooms are caused by over 200 microalgae species, but are most commonly from dinoflagellates, diatoms, raphidophytes, and cyanobacteria, and less commonly from prymnesiophyte, pelagophyte, and silicoflagellate species (Landsberg 2002). Large blooms are often called 'red tides' due to their bright red color (Fig. 1); however, they can also express green, brown, or yellow colors depending on the toxin(s) present and environmental factors. The toxicity of these blooms can affect many local living organisms including humans. Effects can range in severity from acute to extreme, including catastrophic loss of marine life that may result in substantial population changes (Fire *et al.* 2007).

Figure 1. Example of a Harmful Algal Bloom (HAB) Gulf of Mexico, 2013
Harmful Algal Blooms are often referred to as 'red tides' as they visually appear as a red water flow through the ocean waters. *Image courtesy of NOAA (2013).*

The first documented death associated with HABs was in 1793 when a member of the Royal Navy died from shellfish poisoning in an area that is now known as Poison Cove (Lewitus *et al*. 2012). Prior to this written report, Native Americans were aware of the visual keys that signaled problematic conditions. In the Gulf of Mexico area, the local Native Americans would gauge the seasons not by the sun and moon (as many other Native Americans did in other regions of America), but by the presence of fruits ripening and fish dying (Landsberg 2002). In the west coast of Florida, large fish kills would occur in the fall seasonal times, indicating to the Native Americans that winter would be on its way soon. They would also study the waters at night looking for the luminescence of the dinoflagellates indicating the oncoming red tides and mass fish kills.

Karenia brevis, previously known as *Gymnodinium breve*, (Fig. 2) is a dinoflagellate commonly associated with HABs within the coastal waters of the United States. The adverse health effects of *Karenia brevis* (Fig. 2) are caused by brevetoxins (BTXs), which may result in neurotoxic shellfish poisoning (NSP) (Lewitus *et al*. 2012). These neurotoxins are heat-stable, lipid-soluble, polyether compounds that ultimately cause nerve inhibition (Twiner *et al*. 2011).

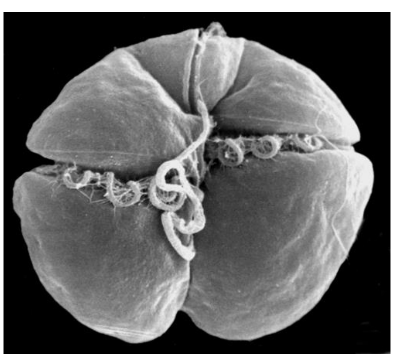

Figure 2. Microscopic photograph of the dinoflagellate, *Karenia brevis*
Scale = 10µm magnification. *Image courtesy of Florida Fish and Wildlife Conservation Commission (2015).*

Toxic aerosols from *K. brevis* can be formed through wave action and may affect both marine mammals and humans through inhalation. To date, no human deaths have been directly linked to neurotoxic shellfish poisoning; however, NSP-related marine mammal deaths and stranding events have been confirmed (Twiner *et al*. 2011). The first suspected aerosol absorption of BTX in a marine mammal was in 1996 when it was found in the lung, liver, and lymphoid tissues of stranded manatees (Bossart *et al.* 1998). NSP is often indirectly caused in marine mammals as it can also be transferred trophically by the consumption of a contaminated food source (Landsberg 2002). NSP can have neurological effects on marine mammals including disorientation and interruption of important reception techniques such as echolocation.

Marine Mammal Strandings

Disturbances in marine mammal health are a main cause of marine mammal strandings. Strandings are defined by the Marine Mammal Protection Act of 1972 as any dead marine mammal on shore or in water, or any helpless live marine mammal including an onshore mammal in need of medical attention or unable to return to its natural habitat (Sea Research Foundation 2011). Most strandings are singular and independent although mass strandings (two or more individuals not including mother/calf) can occur (Jefferson *et al.* 2008).

In the 1980s, the National Marine Fisheries Service (NMFS) created the Working Group for Marine Mammal Unusual Mortality Events (WGMMUME) whose purpose was to establish Unusual Mortality Event (UME) criteria and provide guidance for UME response (Geraci and Lounsbury 2005). A UME is currently defined as a stranding that is unexpected; involves a significant die-off of any marine mammal population; and demands immediate response in accordance with these guidelines (NOAA Fisheries 2015). If a possible UME occurs, the local Operations Center must report it to the NMFS or Fish and Wildlife Services (FWS). These agencies will then investigate the stranding event to determine if it meets UME criteria (Geraci and Lounsbury 2005). Between 1991 and 2015, 62 stranding events have been recognized as UMEs in the U.S., 55% of which impacted cetaceans (Fig. 3). Causation of UMEs varies, however from 1996-2007, there were 12 confirmed UMEs caused by biotoxins (Fig. 4). Research on marine mammal UMEs can provide insight into the overall health of marine environments as well as indicators of future environmental impacts that may have implications on human health (NOAA Fisheries 2015).

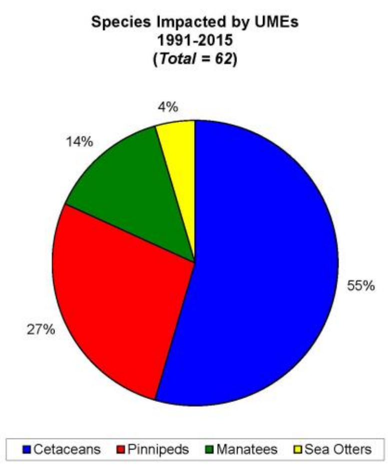

Figure 3. Species Impacted by Unusual Mortality Events in the U.S., 1991-2015
Figure courtesy of NOAA Fisheries (2015).

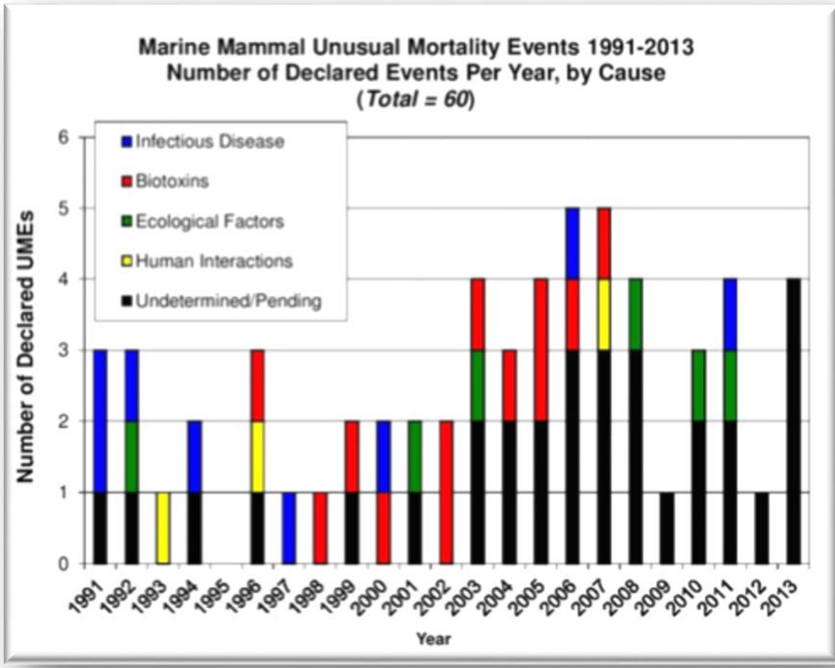

Figure 4. Marine Mammal Unusual Mortality Events 1991-2013
Figure courtesy of NOAA Fisheries (2015).

Linking HABs and Marine Mammal Strandings

Harmful Algal Blooms (HABs) have had dramatic effects on the marine environment and continue to plague ecosystem ecology as well as species interactions, health, and growth. HABs have been shown to affect the marine mammalian realm by contributing to disease, strandings, or both, which ultimately lead to death and species depletion (NOAA 2004; NOAA 2013). Understanding the linkages between HAB events and marine mammal diseases and mortalities may promote conservation awareness and improve HAB prevention policies and monitoring procedures. Marine mammal conservationists may then be able to improve stranding response protocols and, ideally, prevent stranding occurrences.

The objectives of this capstone are to (i) provide an overview of three previously recorded marine mammal stranding events which occurred in Florida during 2004, 2013, and 2014; (ii) create Geographical Information Systems (GIS) maps of environmental data (e.g. *Karenia brevis* concentration levels) and stranding data for these events; and (iii) investigate whether common trends exist among the three case studies which may be used as predictors for future cetacean Unusual Mortality Events. Toxicology results from the 2004 marine mammal mortalities identified the causative agent as *Karenia brevis* (Flewelling *et al.* 2005), thereby directly linking the deaths to that year's HAB. While the toxicology results for the 2013 and 2014 deaths have not yet been completed, *K. brevis* and cetacean stranding data are available for temporal and spatial GIS analyses. Similarities between the 2004 case study, which has been categorized as an UME (Gaydos 2006), and the two latter case studies may allow for the creation of a useful GIS-based stranding prediction tool. Spatial analysis of future HABs may allow stranding first responders to prioritize potential UME locations and stage resources accordingly, thereby minimizing event response times and possibly mitigating HAB-related cetacean mortality.

Case Studies of Cetacean Strandings in Florida

The three case studies reviewed herein were selected because Case Study I is the latest confirmed UME in the western Florida region, and Case Studies II and III were the only subsequent mass mortalities events in the region during which data were collected and available. Stranding data were obtained from the NOAA National Marine Mammal Health and Stranding Response Database and the NOAA SER Marine Mammal

Stranding Database with permission by Elizabeth Stratton of the Southeast U.S. Marine Mammal Stranding Network. HAB data were obtained from the FWC-FWRI's Harmful Algal Bloom Database with permission from the FWC database manager, Dr. Diane Blackwood.

Stranding data collection during each of the case studies was based on a standard categorization of Level A – C, as defined by NOAA necropsy procedures (Twiner 2012). 'Level A' stranding data (species identification, GPS coordinates, gender, estimated life stage via length determination, and carcass condition) were collected for each of the respective cetaceans. Levels of decomposition were recorded according to the standard NOAA necropsy categorization codes (Table 2) (NOAA 2004; Twiner 2012). 'Level B' and 'Level C' data (i.e. necropsy exam and tissue collection) were collected on deceased individuals whenever possible, depending on decomposition level and carcass location. Certain data for Case Studies II and III, such as necropsy results (Levels A, B, and C), were not available for inclusion in this capstone. Stranding details provided for those case studies were acquired during the personal attendance of the author at the strandings and from preliminary NOAA data.

Environmental data included the concentrations of *Karenia brevis* and brevetoxins in the waters proximal to the strandings (Table 1). For this study, *K. brevis* levels above 10,000 cells/L were classified as "elevated" due to the possible effects, including fish kills and other trophic structure mortalities, which could lead to marine mammal strandings.

Table 1. Environmental data classification levels
Data courtesy of Diane Blackwood (personal communication); Twiner (2012).

Classification	***K. brevis*** **Concentration (cells/L)**	**Possible Effects**
Background	<1,000	None anticipated
Very Low	1,000 – 10,000	Possible respiratory irritation
Low	10,000- 100,000	Respiratory irritation; possible fish kills and bloom chlorophyll probably detected by satellites at upper range
Medium	100,000 - 1,000,000	Respiratory irritation and probable fish kills
High	>1,000,000	As above plus discoloration

Case Study I –2004

Overview

An Unusual Mortality Event (UME) involving 107 bottlenose dolphins (*Tursiops truncatus*) occurred between March 10 and April 13, 2004 (NOAA 2004). No live marine mammal strandings were found during this timeframe. Strandings of deceased dolphins were initially clustered but, as time progressed, the spatial locations of individual strandings broadened to span between Escambia and Franklin counties (Fig. 4). The highest density of stranded individuals (~70%) occurred within the St. Joseph Bay area (NOAA 2004). Historically within this region, an average of 21.4 bottlenose dolphin strandings have been recorded per year (Gaydos 2006). The 2004 UME was fivefold greater than the annual average and, thus, became the subject of a large-scale study conducted by NOAA.

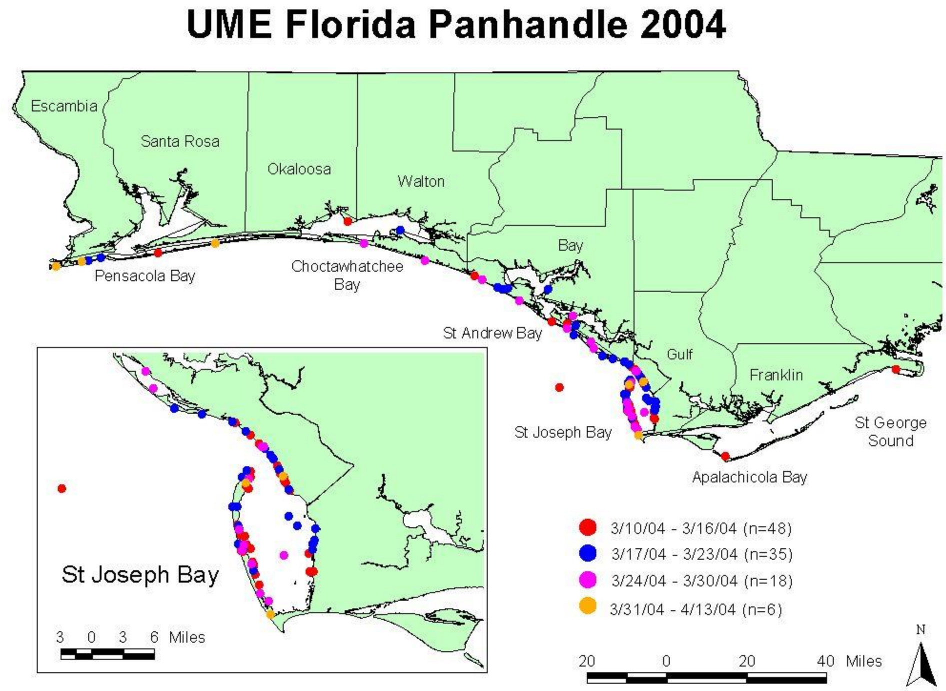

Figure 5. Stranding locations of *Tursiops truncates* during 2004 UME
107 bottlenose dolphins were stranded in the Florida Panhandle. *Image courtesy of NOAA (2004).*

<u>Necropsy Analyses</u>

The specific data gathered from each of the 107 individuals were dependent on the level of decomposition when discovered (Table 2) and ease of access to carcass. Among the stranded individuals, 46 (43%) were males, 36 (34%) were females, and 25 (23%) were unable to be determined due to the level of carcass decomposition (Gaydos 2006). Population demographics included data from neonates (length up to 137 cm) and adults (248 cm or greater) (Twiner 2012).

Table 2. Standard NOAA Decomposition levels of *Tursiops truncatus* during the 2004 UME
Note: One individual could not be categorized. *Data courtesy of NOAA (2004).*

Classification	Decomposition Level	Number of Individuals
Code 1	Live	0
Code 2	Freshly Deceased	19
Code 3	Mildly Decomposed	39
Code 4	Moderately Decomposed	46
Code 5	Severely Decomposed	2

Algal toxin analyses were conducted only on deceased individuals (i.e. decomposition codes 2-5). Level B gross necropsies were performed on 46 (43%) individuals and Level C basic tissue samples were collected from 44 (41%) individuals; together accounting for the 90 (84%) of the total stranded individuals. Seventeen (16%) individuals were unable to be necropsied due to decomposition level, carcass location, or both (NOAA 2004).

In general, most individuals were found to be in relatively good nutritional condition with the exception of a few individuals that expressed slight emaciation (low blubber thickness of <1 cm) (NOAA 2004). In a few of the freshly deceased individuals, sediment was found in the trachea and primary and secondary airways, suggesting the animals may have initially stranded alive and later died on shore which is a common occurrence with mass strandings.

Stomach content analyses indicated the necropsied dolphins were full (mean stomach content of 773 grams) and many of the consumed fish were partially whole, suggesting that the group most likely gorged on prey soon before the strandings occurred (NOAA 2004; Gaydos 2006). The most common identifiable food item within the stomach contents was menhaden (*Brevoortia tyrannus*), a planktivorous fish located within Atlantic waters, including the Gulf of Mexico (Flewelling 2005).

Brevetoxin analyses were conducted via stomach contents, feces, bodily fluids, and tissue samples on 38 of the 46 individuals necropsied (83%) (NOAA 2004; Twiner 2012). Brevetoxins were found most frequently (in 71% of necropsied dolphins) and in the highest concentrations (mean 1800 ng/g) in the stomach contents (Gaydos 2006) (Fig. 5). The levels of brevetoxin in the undigested menhaden found in the stomachs of the dolphins ranged from 136 - 6167 ng/g, with a mean of 2126 ng/g (Twiner 2011). These findings suggest the (i) transfer of brevetoxin trophically from the menhaden to the dolphins and (ii) remote exposure of the dolphins which can be inferred from the low levels of environmental brevetoxins near the stranding sites (Flewelling 2005).

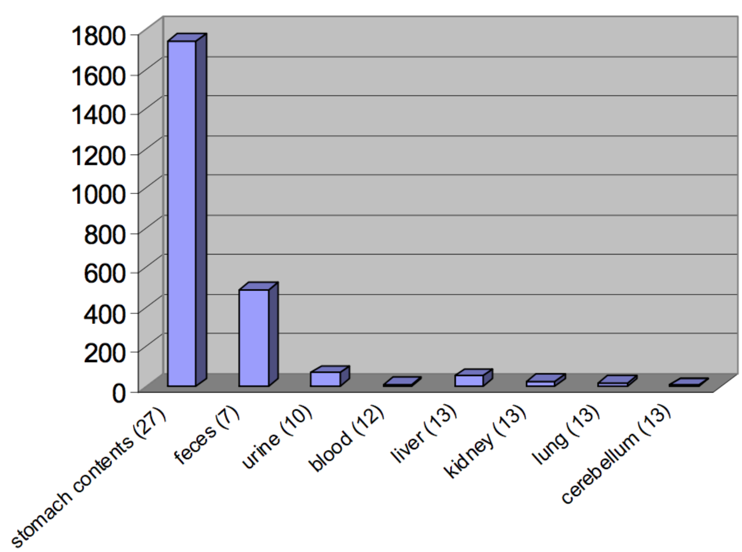

Figure 6. Concentrations of brevetoxins found in necropsy samples during 2004 UME
Vertical scale is ng/g in tissues and feces; ng/ml in urine and blood. *Graph courtesy of NOAA (2004).*

Tissue samples from the blubber, lungs, and liver taken during the necropsies were examined for the presence of pathogens other than brevetoxins. These pathogen results were negative, thereby eliminating other pathogens as possible contributors to the UME (NOAA 2004). The National Marine Fisheries Service (NMFS) officially attributed the 2004 UME to elevated levels of brevetoxins associated with *K. brevis* within the stranded dolphins (NOAA 2004; Gaydos 2006). Environmental data confirmed a spike or bloom in the concentration of *K. brevis* in Sarasota Bay preceding the UME, as summarized below.

Environmental Analyses

Water samples were collected from Sarasota Bay and tested for the presence of *Karenia brevis* (Fig. 6). Of the 235 samples (both surface and subsurface), only two indicated *K. brevis* presence, both of which were categorized as 'low' level (<1000 cells/L) (Twiner 2012). Brevetoxins were present in 15% of the respective water samples (n= 82), all of which had 'low' concentrations (<2 μg/L) (Twiner 2012). 75% of the samples which tested positive for brevetoxin were collected from the same geographic area within Sarasota Bay, suggesting that a localized bloom may have occurred in the Sarasota Bay area prior to the strandings (Twiner 2012). The occurrence of a bloom is supported by higher than normal levels of *Karenia brevis* in Sarasota Bay during February, one month before the start of the stranding event (Fig. 6). Satellite imagery confirms that high concentrations of chlorophyll A were present along the coast of the Florida panhandle approximately 1-2 months prior to the strandings (Fig. 7), which supports the presence of an algae bloom; however, the flora responsible for the chlorophyll A blooms cannot be determined from these images.

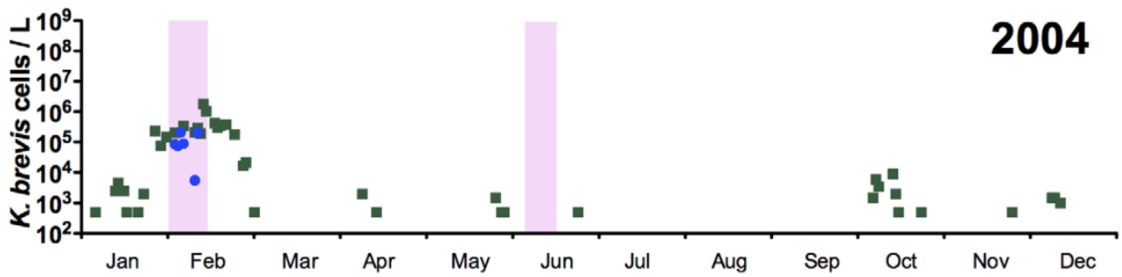

Figure 7. *Karenia brevis* cell counts in the Sarasota Bay during 2004
Peak *K. brevis* concentrations occurred in February, one month before the *Tursiops truncates* UME. Purple areas in February and June represent times during which dolphin health assessments were conducted. *Graph courtesy of Twiner (2011).*

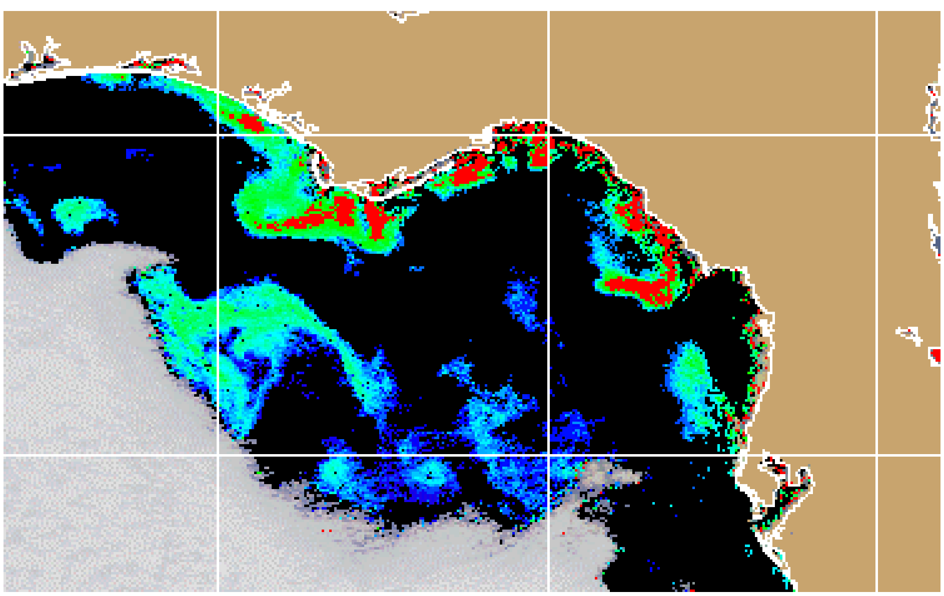

Figure 8. Chlorophyll A satellite imagery of the Florida panhandle on February 28th, 2004
High chlorophyll a concentrations are shown in red and indicate the spatial extent of the coastal algae bloom. *Image courtesy of NOAA (2004).*

Throughout 2004, environmental levels of *K. brevis* concentrations remained below the elevated threshold (<10,000 cells/L) which is associated with mass mortalities of fish and other marine species. Waterborne *K. brevis* concentrations increased 1-3 orders of magnitude during February 2004, approximately one month before the UME. These results support the transfer of brevetoxin trophically from the environment to the menhaden (*Brevoortia tyrannus*) and subsequently to the dolphins (*Tursiops truncates)*, eventually reaching lethal levels.

Case Study II – Florida 2013

Overview

The second case study occurred on December 3rd, 2013 in the southern region of the Florida Everglades within the Homestead area of Monroe County, FL. Case Study II is referred to as a mass stranding event rather than as a UME as it has not yet been confirmed by the appropriate agencies. This event involved 51 short-finned pilot whales *(Globicephala macrorhynchus)* (NOAA 2013).

Necropsy Analyses

29 (57%) of the stranded whales were still alive (Code 1) but were subsequently considered ‘missing’ after returning to open ocean and having no documented resurfacing in the following weeks. The remaining 22 stranded whales were recently deceased with Code 2 decomposition. The event was reported very early and stranding response teams were able to rapidly respond to each stranded individual or group. Seasonal

environmental conditions (e.g. lower temperatures, lack of humidity, reduced sun exposure) contributed to slower decomposition rates.

Gross necropsies were performed on 16 (73%) of the deceased whales including organ tissue samples for cryogenic and histologic testing (e.g. skin, lung, liver, heart, kidney, brain, pyloric glands, mesenteric lymph nodes), and non-organ samples (e.g. spinal fluid, blubber, stomach contents) for subsequent pathological testing. These samples are currently undergoing biotoxin analysis; however, results were not yet available at the time of this review. NOAA responders are hopeful that the pathology results will identify causative toxin(s) for the stranding event and associated mortalities.

Environmental Analyses

In order to be consistent with Case I, *Karenia brevis* data collected three months prior to the stranding events are reviewed herein. Of the 222 samples, 16 (7%) indicated elevated *K. brevis* presence. Within this subset of elevated samples, 11 were 'low' (10,000 – 100,000 cells/L), three were 'medium' (100,000 – 1,000,000 cells/L), and two were 'high' (>1,000,000 cells/L).

GIS Map of Environmental and Stranding Data

A stranding map for Case Study II, similar to Figure 4, has not yet been published by NOAA. Therefore, a GIS map was created for the purposes of this capstone using the coordinates of the HAB sampling points and elevated *K. brevis* results relative to the stranding location (Fig. 8). Elevated *K. brevis* levels were first recorded in the November 18, 2013 samples, 107.76 km northwest (319°) from the initial stranding (Table A1).

Elevated concentrations of *K. brevis* were observed to spread northwest through November 26, 2013, the sample set immediately preceding the first stranding on December 3, 2013. The HAB had dissipated and *K. brevis* concentrations were no longer elevated by the time the first pilot whale stranding was reported.

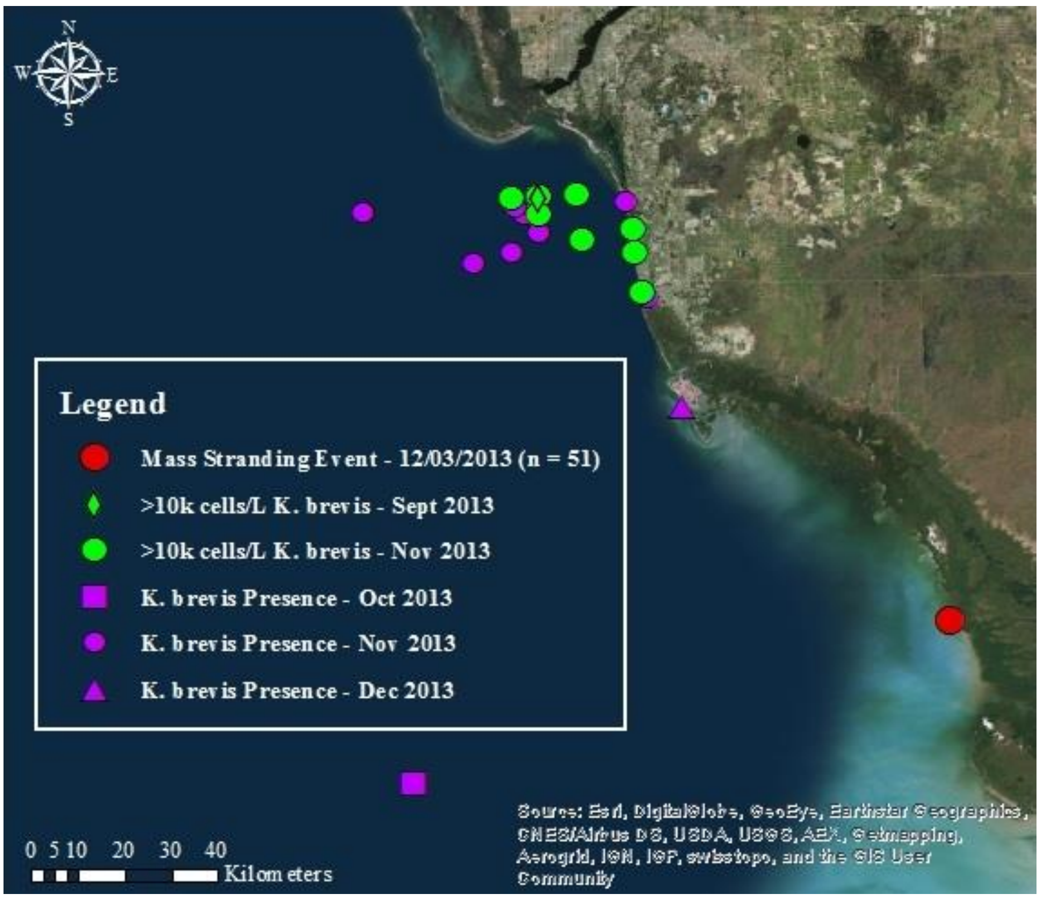

Figure 9. GIS Map of Case Study II Mass Stranding Event and HAB Presence
GPS coordinates show the mass stranding event of Case II in red, elevated *K. brevis* levels in green, and the presence of *K. brevis* below elevated levels in purple.

Case Study III – Florida 2014

Overview

The third case study occurred on January 19th, 2014 in the Everglades National Park region of Naples, Collier County, FL. Currently, Case Study III has not been labeled an official UME; however, much like Case Study II, it is considered a mass stranding event of 25 short-finned pilot whales *(Globicephala macrorhynchus)* (NOAA 2013). Upon arrival of stranding response teams, 19 individuals were live and six were deceased with varying levels of decomposition. Relocation was required for the necropsies to be performed on the six carcasses due to remote location and/or media presence. The relocation process may have caused additional decomposition to occur pre-necropsy. The remaining Code I (live) individuals were guided to open ocean and considered 'missing' after no additional sightings in the immediate area were reported in the weeks following. All carcasses were identified as Code 2 (freshly deceased). Full necropsies (Levels A, B, and C) were conducted on five of the six carcasses and one limited (Level A only; most likely due to the relocation process). The rescue response for this event was less than 24 hours, enabling necropsies to be conducted within 72 hours. Seasonal environmental conditions (e.g. lower temperatures, lack of humidity, reduced sun exposure) contributed to slower decomposition rates.

Necropsy Analyses

All six necropsied individuals were adults consisting of one male, four females, and one pregnant female (NOAA 2013). Complete necropsies of the individuals included cryogenic and histologic samples which will be pathologically tested. Samples for further testing included skin, liver, lung, heart (first and second chambers, endocardium), kidney, urinary bladder, mesenteric lymph node, spleen, testes, brain (cerebellum and vortex), blowhole swab, urine, anus, intestines, teeth, and stomach contents. Where present, cysts were also removed and collected. While none of the individuals were categorized as 'emaciated,' most had empty stomachs with only fluid present. A further necropsy was conducted on the fetus within the pregnant female, with basic samples collected (all available due to size). All samples were sent for biotoxin analysis; however, at the time of this writing, the results are not yet available. Level A data were collected on the single individual which was not fully necropsied.

Environmental Analyses

Similar to the previous case studies, *Karenia brevis* data collected three months prior to the stranding events are discussed herein. Of the 217 samples, 16 (7%) indicated elevated *K. brevis* presence. Within this subset of elevated samples, 11 were 'low' (10,000 – 100,000 cells/L), three were 'medium' (100,000 – 1,000,000 cells/L), and two were 'high' (>1,000,000 cells/L).

GIS Map of Environmental and Stranding Data

Case Study III occurred six weeks after Case Study II; however the same *K. brevis* elevated levels were observed and no additional elevations in the non-overlapping timeframe were recorded. A second GIS map was created for the purposes of this capstone using these elevated *K. brevis* data and the event III mass stranding data (Fig.

9). Elevated *K. brevis* levels were recorded between November 18 and November 26, the closest of which was just 3.99 km north (347°) from the stranding (Table A2).

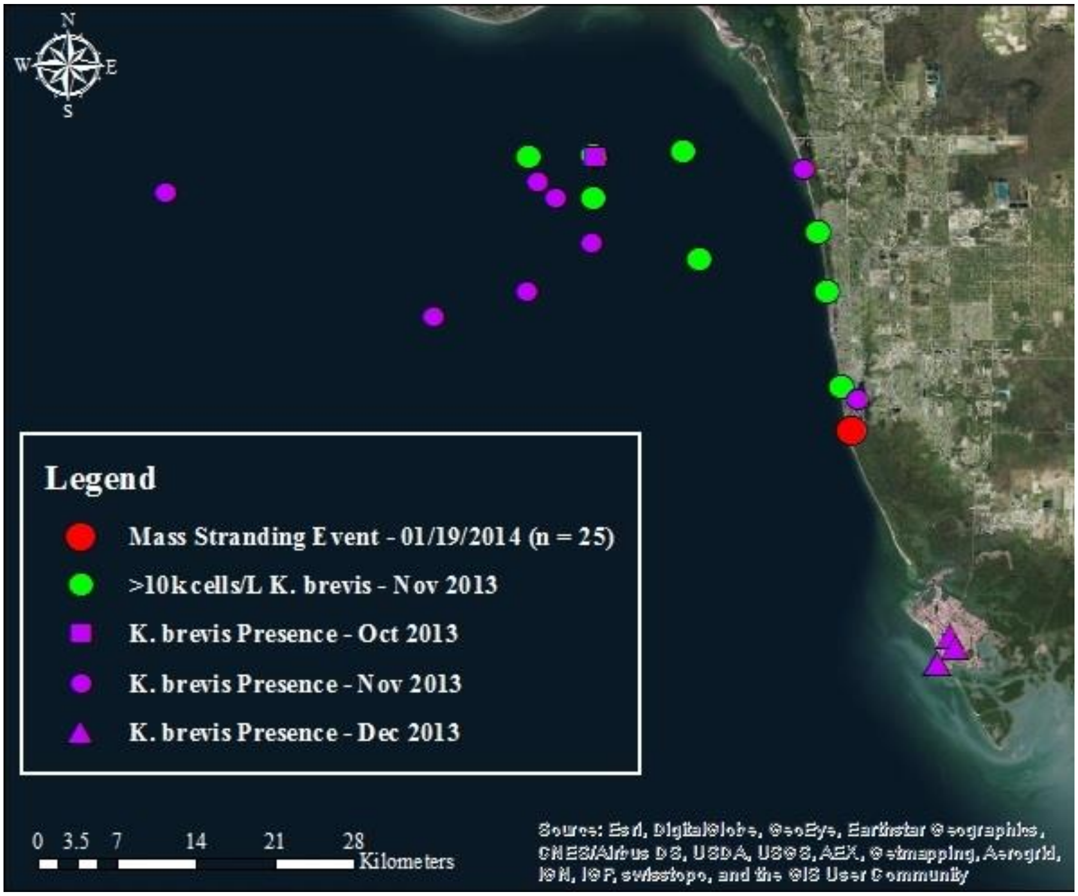

Figure 10. GIS Map of Case Study III Mass Stranding Event and HAB Presence
GPS coordinates show the mass stranding event of Case II in red, elevated *K. brevis* levels in green, and the presence of *K. brevis* below elevated levels in purple.

Discussion

Spatial and temporal similarities between the confirmed UME of Case Study I, and the ‘unconfirmed’ events of Case Studies II and III may be used as predictors for future cetacean strandings and UMEs, leading to more efficient rescue responses. The 2004 UME event has been positively linked to *K. brevis* exposure, via toxicology results, by the presence of BTX in the tissues of the stranded cetaceans (NOAA & FWC 2004). This event is used as a benchmark for comparison to the two subsequent events for which toxicology results are pending. Environmental data (e.g. HAB presence) and locations of mass strandings, which are available for each of the case studies, are used for the spatial and temporal comparisons described below. The possibility of the two unconfirmed cases becoming UMEs is still present as further results are pending.

Spatial Comparisons

Each of the three Case Studies discussed herein occurred on the Western coastal region of Florida. The confirmed UME (Case Study I) occurred in the northwest panhandle region of St. Josephs Bay. Case Studies II and II occurred in Monroe and Collier Counties, near the Florida Everglades, approximately 800 km and 700 km south of Case Study I, respectively. Case Studies II and III were separated by 88 kilometers.

Case Study I involved bottlenose dolphins (*Tursiops truncatus*) whereas Case Studies II and III involved short-finned pilot whales *(Globicephala macrorhynchus).* The different cetacean species may have distinct migration patterns, which may account for the significant spatial separation between the events. Interestingly, the *T. truncatus* strandings were recorded across approximately 257 km of coastline whereas the *G.*

macrohynchus strandings occurred within tight clusters (i.e. < 3 km spatial range) during both events; the cause(s) of these dissimilarities remain(s) unknown.

Unfortunately, the distance between the Case Study I UME and elevated *K. brevis* levels could not be exactly pinpointed because the GPS coordinates for the *K. brevis* measurements were not available and only noted as being taken in the Sarasota Bay area (Twiner 2012). GPS data points are now collected by the Florida Fish and Wildlife Conservation Commission: Fish and Wildlife Research Institute and were, therefore, available during environmental sampling for Case Studies II and III (Diane Blackwood, Personal Communication). Because data are missing for Case Study I, spatial patterns between the respective elevated *K brevis* concentrations and the stranding locations may only be compared for Case Studies II and III, presuming that *K. brevis* will be shown to be the causative agent of these mortalities. The HAB bloom (shown by elevated *K. brevis* data) was located in a northwest direction from both Case Study II and III stranding sites. In general, the HAB first occurred northwest of the stranding sites and flowed closer in a southeast direction covering 31.65 km.

All elevated *K. brevis* results for Case Study II occurred 90-125 km northwest (within 6°) of the stranding site (Table A1). 87% of Case Study III elevated levels occurred 19-36 km northwest (within 8°) of the respective stranding site (Table A2). These data may be used to improve response times to strandings: if elevated *K. brevis* levels are detected among the Collier County sample locations, stranding response kits should be staged between Algiers Beach and Naples Pier. Marine mammal response organizations in these areas should also be notified in order to create an on-call rescue team; providing localized planning time for interference such as weather, media, traffic, and equipment supply inventory.

Case Study I linked the cause of the bottlenose dolphin strandings to lethal *K. brevis* toxin levels transferred trophically from their diet of menhaden. Spatial patterns connecting the menhaden to *K. brevis* exposure is unknown. Similar links to specific food sources are not yet available for the pilot whales for the latter Case Studies. Case Study III may be difficult in proving trophic links because, while the liquid deposits can be tested for BTX presence, most of the stomachs were empty of solid prey. Should the trophic link for the transmission of *K. brevis* toxins to pilot whales be identified, the spatial patterns of these prey may provide useful insight for narrowing the range of projected stranding events.

Further studies are recommended which compare the spatial patterns of cetacean migrations, menhaden and other prey species distributions, and HAB dispersion.

Temporal Comparisons

Case Study I involved *Tursiops truncates* during the spring months between March and April, 2004. Case Studies II and III involved *Globicephala macrorhynchus* during the winter months of December 2013 and January 2014, respectively. Elevated levels of *K. brevis* were first detected 2-9 weeks before the two latter strandings and decreased to non-lethal levels the day before (December 2nd, 2013) the first pilot whale death. While the elevated *K. brevis* results are similar for both whale stranding events, the overall *K. brevis* presence differs. Three additional *K. brevis* presence detections occurred in December, after the Case Study II event and before the Case Study III event. Two of these detections were on December 3rd, 2013 the day of the Case Study II event. The average distances from these detections to event sites for Case Studies II and III were 69.45 km northwest (same day as event) and 20.28 km northwest (7 weeks prior to event), respectively. Although not included in the GIS mapping, which included data prior to the events, the presence of *K. brevis* was detected on January 24th 2014, three days after the Case Study III event. The closest temporal elevated *K. brevis* results occurred November 26th, 2013, one week prior to the Case Study II event and eight weeks prior to the Case Study III event. Elevated *K. brevis* readings may be used to assist in temporal predictions of cetacean strandings, forewarning responders to be on alert.

Further studies are recommended which compare the temporal patterns of cetacean migrations, spawning aggregations of menhaden and other prey species, and harmful algal blooms.

Conclusions

The objectives of this research were met in that (i) three recorded mass cetacean stranding events were explained in detail for an overall understanding, (ii) GIS maps were created in order to observe correlations of the three mass mortality events, and (iii) trends between the three stranding events were examined in order to produce predictors for first responders to use in creating more efficient response procedures.

GIS analysis can aid in the development of a cetacean stranding prediction tool by providing visual models of temporal and spatial trends that compare mass mortality locations to causative factors. In order to solidify any correlations between *K. brevis* presence and cetacean mass strandings, additional research would need to be conducted including possible confounding factors such as climate variability, localized weather patterns, anthropogenic stressors, cetacean migration patterns, and ocean currents among possible others. However, if *K. brevis* is found to be the causative agent for the pilot whale strandings in Case Studies II and III, as it was in Case Study I, common spatial and temporal factors may allow stranding first responders to prioritize potential UME locations and stage resources accordingly. Further research would need to be conducted through the various abiding organizations in order to label Case Studies II and III as UMEs.

Based on the GIS analysis described herein, elevated *K. brevis* levels (>10,000 cells/L) in Sarasota Bay during November may be predictive of pilot whale stranding events in the following 2-9 weeks in the Everglades region between Algiers Beach and Naples Pier. Presuming similar temporal correlations, elevated *K. brevis* readings in Sarasota Bay during January or February may be predictive of bottlenose dolphin strandings in March or April in the Panhandle region between Pensacola and Apalichicola Bays. During these alert timeframes, strandings kits may be staged within 125 kilometers of an initial elevated *K. brevis* finding and on-call schedules for responders may be created. Such initiatives could allow for quicker response times and closer supply kit availability, thereby, increasing live mammal rescue – a primary goal of marine mammal rescue and response teams.

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Appendices

GPS coordinates for the HAB sampling points and mass stranding locations for Case Studies II and III were plotted using ArcGIS to create Figures 8 and 9, respectively. Subsets of these data, specifically the locations of elevated HAB samples relative to the mean stranding location are provided in Tables A1 and A2.

Table A1. Elevated *K. brevis* Locations Relative to Case Study II Mass Stranding
The event location depicts the mean GPS location of the 51 short-finned pilot whales *(Globicephala macrorhynchus)* stranded on 12/03/2013.

	Date	Longitude	Latitude	Distance (km)	Degree	
EVENT II – Homestead, Monroe County, FL	12/03/13	25.499880	-81.211800			
K. brevis Sampling Points (>10,000 cells/L)						
	11/18/13	26.232783	-81.918630	107.76	319	NW
	11/18/13	26.314100	-82.002020	120.02	318	NW
	11/18/13	26.314100	-82.002020	120.02	318	NW
	11/18/13	26.314100	-82.002020	120.02	318	NW
	11/18/13	26.314100	-82.002020	120.02	318	NW
	11/22/13	26.315960	-82.002470	120.21	318	NW
	11/22/13	26.315960	-82.002470	120.21	318	NW
	11/22/13	26.318700	-81.930980	115.85	321	NW
	11/22/13	26.318700	-81.930980	115.85	321	NW
	11/22/13	26.280870	-82.003110	117.36	317	NW
	11/22/13	26.280870	-82.003110	117.36	317	NW
	11/25/13	26.314090	-82.054810	123.58	317	NW
	11/25/13	26.314090	-82.054810	123.58	317	NW
	11/26/13	26.131630	-81.806300	91.93	319	NW
	11/26/13	26.207300	-81.816900	99.10	322	NW
	11/26/13	26.253750	-81.823770	103.62	323	NW
Average				114.78	318.81	NW

Table A2. Elevated *K. brevis* Locations Relative to Case Study III Mass Stranding
The event location depicts the mean GPS location of the 25 short-finned pilot whales *(Globicephala macrorhynchus)* stranded on 01/19/2014.

	Date	Longitude	Latitude	Distance (km)	Degree	
EVENT III - Naples, Collier County, FL	01/19/14	26.096478	-81.797420			
K. brevis Sampling Points						
	11/18/13	26.232783	-81.918630	19.36	321	NW
	11/18/13	26.314100	-82.002020	31.61	319	NW
	11/18/13	26.314100	-82.002020	31.61	319	NW
	11/18/13	26.314100	-82.002020	31.61	319	NW
	11/18/13	26.314100	-82.002020	31.61	319	NW
	11/22/13	26.315960	-82.002470	31.80	319	NW
	11/22/13	26.315960	-82.002470	31.80	319	NW
	11/22/13	26.318700	-81.930980	28.01	331	NW
	11/22/13	26.318700	-81.930980	28.01	331	NW
	11/22/13	26.280870	-82.003110	28.98	314	NW
	11/22/13	26.280870	-82.003110	28.98	314	NW
	11/25/13	26.314090	-82.054810	35.26	313	NW
	11/25/13	26.314090	-82.054810	35.26	313	NW
	11/26/13	26.131630	-81.806300	3.99	347	N
	11/26/13	26.207300	-81.816900	12.43	350	N
	11/26/13	26.253750	-81.823770	17.62	351	N
Average				26.75	324.94	